CERTIFICATE OF MAILING

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ELI LILLY AND COMPANY

By James Kelly Date 2/18/03

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	James A. Hoffmann & Jirong Lu	)
Serial No.	:	09/928,198	)
Filed	:	August 10, 2001	) Group Art Unit: 1646
For	:	FSH Formulation	) Examiner: R. DeBerry
Docket No.	:	X-12383N	)

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Arlington, VA 22202
Sir:

I, Ranmali Wijayaratne, hereby state and declare that:

1. I hold the degree of Doctor of Philosophy in Chemistry from the University of Maryland, College Park (1982).
2. I am currently a Research Scientist at Lilly Technology Center (Lilly), Indianapolis, Indiana. I have been a scientist with Lilly since February 1994 and have been involved with chromatographic analysis for numerous protein formulations.
3. I am co-author of 12 scientific publications in refereed journals.
4. I am not named as an inventor on any US patents or patent applications, including the present application.

5. My curriculum vitae is attached.

6. Attached are data from an experiment performed in a laboratory under my supervision. The goal of this experiment was to determine the effect of benzyl alcohol on the stability of a formulation that contained an FSH variant that differed from human FSH only in lacking the last three amino acids of the beta subunit. The experimental design was to examine one factor with the others being fixed. The stability indicating assay utilized was size exclusion chromatography.

7. The formulations included the following ingredients in an aqueous diluent:

   Fixed factors: 50 μg/mL FSH variant, 15 mM citrate, 5.0% sucrose, 0.1% methionine, pH 7.0.

   Varied factor: 0.0% benzyl alcohol and 0.9% benzyl alcohol

8. The vials were stored at temperatures of 5°C, 25°C and 40°C, which represent the following three levels of storage temperature:

   Refrigerated conditions at 5°C (typically 2-8°C),

   Room temperature conditions at 25°C (typically between 20-25°C), and

   Accelerated conditions at 40°C (typically 37°C or above).

9. Samples were pulled from each vial at 0, 1, 2, 3, 4, 5, 7, 8, 10 and 12 weeks, and were analyzed for heterodimer content using size exclusion chromatography.

10. The size exclusion chromatographic (SEC) assay is stability indicating and is a measure of the % heterodimer, % subunits and the chemical potency (μg/mL). Duplicate injections into the HPLC were made from each vial at each time point. The HPLC instrumentation included HP1100 chromatography systems and the HP1000 chromatography data collection system.

11. The SEC-HPLC ambient assay conditions were as follows: Column type: TSK-GEL G2000SWXL, Flow Rate = 1.0 mL/min; Elution buffer = 0.1 M sodium phosphate, pH 7.4 (95%) and 5% isopropyl alcohol; temperature = ambient. UV detection was performed at 214 nm.

12. The assay method can readily detect heterodimer instability, and was validated according to ICH guidelines for Phase I clinical trials in terms of linearity, precision (repeatability), specificity, detection limit and quantitation limit, range, and accuracy.

13. Results for the experiment are shown in Table A.

Table A. Percent Heterodimer in FSH Formulation vs. Time and Temperature.

	40°C		25°C		5°C	
Weeks	FSH control	FSH + BzOH	FSH control	FSH + BzOH	FSH control	FSH + BzOH
0	96.19	96.42	96.19	96.42	96.19	96.42
1	95.67	94.21	-	-	-	-
2	94.31	89.55	96.28	95.89	-	-
3	93.58	88.53	-	-	-	-
4	93.48	88.37	96.49	96.57	96.51	96.5
5	93.2	90.2	-	-	-	-
7	-	-	95.78	95.18	-	-
8	-	-	95.78	95.20	95.83	95.66
10	-	-	95.99	95.44	-	-
12	-	-	-	-	96.09	95.79

From the data in Table A, I conclude that

14. At storage conditions 5°C and 25°C, the initial, 2, 4, 7, 8 and 12-week time point data for 0.0% Benzyl alcohol and 0.9% Benzyl alcohol were about the same with regards to percent heterodimer. Small differences observed were within the margin of error in the experimental method. There was significant degradation at 40°C at 2 weeks and beyond for samples with and without benzyl alcohol, but a faster rate of degradation with benzyl alcohol than without it.

15. The FSH variant formulation with benzyl alcohol is stable at both refrigerated temperature and at room temperature.

16. The FSH variant formulations with benzyl alcohol are pharmaceutically acceptable formulations that would be suitable for multiple dosing at both room temperature and at refrigerated temperature.

17. The FSH variant formulations with benzyl alcohol are sufficiently stable, with respect to heterodimer content, to provide a multi-dose pharmaceutical product.

18. The rate of heterodimer loss at room temperature is about the same with benzyl alcohol as without it, at both room temperature and at refrigerated temperature.

19. The presence of benzyl alcohol in these formulations permits FSH to be made available in pharmaceutically acceptable, multi-dose forms suitable for use at room temperature.

20. These data obtained with an FSH variant are representative also of the heterodimer stability of human FSH with benzyl alcohol.

I further declare that:

21. It would have been difficult to predict the effect of benzyl alcohol on stability of FSH formulations. If any prediction could have been made, it would have been that as temperature increased, any loss of heterodimer due to benzyl alcohol would likewise increase with temperature.

22. At 40 °C, benzyl alcohol accelerates heterodimer loss compared with the control lacking benzyl alcohol, whereas, at refrigerated temperature (5 °C) and at room temperature (25 °C), the stability of formulated FSH with and without benzyl alcohol is about the same.

23. I would have expected the rate of heterodimer loss in the FSH formulation containing benzyl alcohol at room temperature to be greater than what was actually observed, that is, to be more similar to the rate of loss observed with the FSH + benzyl alcohol formulation at (40 °C) than to the rate of loss observed at 5 °C.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Ranmali Wijayaratne

Ranmali Wijayaratne, Ph.D.

2/18/2003

February 18, 2003

CURRICULUM VITAE

RANMALI D. WIJAYARATNE

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Carmel, IN 46033

PHONE: (317)- 277-2744 (Office)

EDUCATION/LICENSES

1973 B.S. (Chemistry-Honors) University of Sri Lanka, Colombo, Sri Lanka
1977 M.S. (Analytical Chemistry) North Carolina State University, Raleigh, NC
1982 Ph.D. (Analytical Chemistry) University of Maryland, College Park, MD
1993 MBA (with distinction) Keller Graduate School of Management, Chicago, IL

EXPERIENCE

2000- To Present - **Analytical Team leader, Pharmaceutical Product Development, Eli Lilly Co., Indianapolis, IN**
Responsibilities include being the Team Leader representing all analytical activities on several protein or small molecule project(s). In addition to being on the "core" program team, directs the analytical activities to support the development of drugs through IND to NDA phases. Has developed excellent relations with Discovery and other functional areas by working in an interdisciplinary environment through good teamwork and communication and writing skills.

1998-2000 - **Development Project Manager, Lilly Corporate Center, Eli Lilly & Co, Indianapolis, IN**
Performed duties as the CM&C project Manager (Chemistry, Manufacturing and Control) for two early decision phase drug products. Project goals were met though coordinating and managing the CM&C functions of bulk manufacturing, analytical , formulation, regulatory, CT operations and included dealing with external partners.

1994-1998 - **Research Scientist, Research Technologies and Proteins, Eli Lilly and Co, Indianapolis ,IN**
Responsibilities included planning, organizing, and directing the activities of an analytical laboratory unit supporting fermentation and purification efforts of bulk drug development. These included developing robust and rugged methods for in-process assays with appropriate validation criteria and transfer to QC.

1987-1993	**Head, Chromatography, Searle, Skokie, Il** Responsibilities included staffing and directing the an analytical laboratory unit supporting the discovery of new targets. Supervised technical personnel and had extensive "hands on" experience with modern technology in the characterization of small and large molecules.
1984-1987	**Research Investigator, Searle, Skokie, Il**
1982-1984	**NSF Post Doctoral Fellowship; National Center for Atmospheric Research, Boulder, Co**

Knowledge/Skills/Abilities/Strengths

Knowledge

- Analytical quality systems/method development
- Analytical protocol development, SOPs, method validation, knowledge of GMP and regulatory requirements
- Development Project Management and knowledge of overall drug development
- Familiarity with core therapeutic areas as it pertains to Discovery/Development
- Business Alliances; In addition to writing term sheets for contractual agreements knowledge of alliance management to effectively deal with third parties; Includes assessment of potential licensing opportunities (due diligence).

Skills/Abilities /Strengths:

- Good leadership skills; good scientific reasoning skills & problem solving skills;
- Good interpersonal skills; coaching and communication skills
- Good conflict resolution skills, negotiation skills and project management skills
- Ability to bring a business perspective to a scientific endeavor

Publications:
12 publications in refereed journals and 15 internal technical documents

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ELI LILLY AND COMPANY

By James J Kelley Date 2/18/03

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	James A. Hoffmann & Jirong Lu	
Serial No.	:	09/928,198	
Filed	:	August 10, 2001	Group Art Unit: 1646
For	:	FSH Formulation	Examiner: R. DeBerry
Docket No.	:	X-12383N	

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Arlington, VA 22202
Sir:

I, John M Beals, declare that:

1. I hold the degree of Ph.D. in Biochemistry from the University of Notre Dame, Notre Dame, Indiana (1987).

2. I have been employed since 1990 by Eli Lilly and Company in the following capacities: Sep, 1990 to Oct, 1994: Senior Pharmaceutical Chemist; Oct, 1994 to Jan, 1999: Research Scientist; Jan, 1999 – present: Senior Research Scientist, Group Leader. In these positions, I have been responsible for, among other things, research on protein-excipient interactions in formulations and their influence on protein formulation stability, structural properties of proteins for NDA submission, pre-formulation characterization of a new protein drug candidate, and technical direction

of research efforts on internal bioproduct opportunities associated with diabetes, inflammation, cardiovascular disease, dementia, autoimmune disease, and hematopoiesis.

3. I have authored or co-authored twenty-six (26) publications in reviewed journals.

4. I am the inventor or co-inventor of several pending United States patent applications, and no issued US patents.

5. I am not an inventor named in this application.

6. I have reviewed the application and the Office Action.

7. My Curriculum vitae is attached.

FSH PRODUCTS

I further declare that:

8. At the time that the presently claimed invention was made, none of the following FSH products contained a preservative, let alone benzyl alcohol. I am not aware of the composition of any other FSH product at the time that the invention was made.

Pergonal

9. From at least as early as 1975, Serono marketed an FSH product, called Pergonal, which contained "a purified preparation of gonadotropins extracted from the urine of postmenopausal women."[1] Pergonal was supplied as an ampoule that contained 75 IU of FSH and 75 IU of LH plus lactose in a lyophilized form.[2] To administer the product, the user was instructed to "[d]issolve the contents of one ampule of Pergonal

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1 Physician's Desk Reference ("PDR"), 29th Ed., 1975, pages 1366 – 1367 (Reference CAS).

2 Reference CAS, PDR 29th Ed., page 1367.

in one to two ml. of sterile saline and administer intramuscularly immediately." Any unused reconstituted material was to be discarded.[3]

10. Three other PDR excerpts likewise indicate that Pergonal contained a mixture of FSH and LH, that the lyophilized material was to be reconstituted with a saline diluent, then used immediately, and any remaining discarded.[4] None of the cited PDR excerpts mention that Pergonal contained a preservative, but they all clearly indicate that Pergonal was a single-use product.

11. Pergonal was an FSH product that did not contain a preservative from the time of its launch in the 1960s until the time that the present invention was made.

12. Pergonal was a single-use product that needed to be reconstituted each time it was to be used.

13. Dosing with Pergonal occurred daily for between seven (or nine) and twelve days, followed by a dose of human chorionic gonadotropin (hCG),[5] which is also a heterodimeric gonadotropin.

14. As mentioned elsewhere herein, most if not all hCG products on the market at the same time as Pergonal contained a preservative (usually benzyl alcohol) and were multi-dose products.

15. Even though the recommended course of therapy with Pergonal required multiple dosings over a period of from seven to twelve days, and even though Serono, the company that manufactured and marketed Pergonal, also manufactured and marketed an hCG product (Profasi) in a preserved multi-dose form during most of the period

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3 Id.

4 PDR, 49th Ed., 1995, pages 2325 – 2327 (Reference CAT); PDR, 51st Ed., 1997, pages 2618 – 2620 (Reference CAU); and PDR, 54th Ed., 2000, pages 2946 – 2947 (Reference CAV).

5 Reference CAS, page 1367; Reference CAT, page 2327; Reference CAU, page 2620; Reference CAV, page 2947.

that Pergonal was on the market, Serono never provided Pergonal as a multi-dose product.

Humegon

16. Organon marketed an FSH product, called Humegon, beginning in about 1983. Like Serono's Pergonal, Humegon was a purified preparation of gonadotropins extracted from the urine of postmenopausal females that contained both FSH and LH activity.[6] Each vial of Humegon contained either 75 or 150 IU of FSH activity and 75 or 150 IU of LH activity, plus lactose and phosphate salts in a sterile, lyophilized form.[7] The user was instructed to dissolve the contents of one vial of Humegon in one to two mL of sterile saline, to administer intramuscularly immediately, and to discard any unused reconstituted material.[8] Therefore, Humegon was an FSH product that did not contain a preservative from the time of launch in about 1983 until the time that the present invention was made.

17. Humegon was a single-use product that needed to be reconstituted each time it was to be used.

18. Dosing with Humegon occurred daily for between seven and twelve days, followed by a dose of human chorionic gonadotropin (hCG),[9] which is also a heterodimeric gonadotropin.

19. As mentioned elsewhere herein, most if not all hCG products on the market at the same time as Humegon contained a preservative (usually benzyl alcohol) and were multi-dose products.

20. Even though the recommended course of therapy with Humegon required multiple dosings over a period of from seven to twelve days, and even though Organon, the

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6 PDR, 54th Ed., 2000, pages 2095 – 2097 (Reference CAW).

7 Id., page 2095.

8 Id., pages 2096 – 2097.

9 Id., page 2096.

company that manufactured and marketed Humegon, also manufactured and marketed an hCG product (Pregnyl) in a preserved multi-dose form during most of the period that Humegon was on the market, Organon never provided Humegon as a more convenient, multi-dose product.

Metrodin

21. Serono marketed an FSH product called Metrodin beginning, I believe, in about 1984, and a related FSH product called Metrodin HP beginning, I believe, in about 1993. Metrodin was a preparation of gonadotropin extracted from the urine of postmenopausal women that contained either 75 or 150 IU of FSH activity and lactose in a sterile, lyophilized form.[10] The user was instructed to dissolve the contents of one ampule of Metrodin in one to two mL of sterile saline, to administer intramuscularly immediately, and to discard any unused reconstituted material.[11] Metrodin was an FSH product that did not contain a preservative from the time of launch in about 1984 until the time that the present invention was made.

22. Metrodin was a single-use product that needed to be reconstituted each time it was to be used.

23. Dosing with Metrodin occurred daily for at least seven days using from one to four vials per day, followed by a dose of human chorionic gonadotropin (hCG),[12] which is also a heterodimeric gonadotropin.

24. As mentioned elsewhere herein, most if not all hCG products on the market at the same time as Humegon contained a preservative (usually benzyl alcohol) and were multi-dose products.

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10 PDR, 51st Ed., 1997, pages 2616 – 2618 (Reference CAX).

11 Id., page 2618.

12 Id.

25. Even though the recommended course of therapy with Metrodin required multiple dosings over a period of from seven to twelve days, and even though Serono, the company that manufactured and marketed Metrodin, also manufactured and marketed an hCG product (Profasi) in a preserved multi-dose form during the period that Metordin was on the market, Serono never provided Pergonal as a more convenient, multi-dose product.

Fertinex

26. Serono launched a single-dose FSH product called Fertinex in the 1990s that contained a highly purified FSH extracted from the urine of post-menopausal women and lactose in sterile saline after reconstitution.[13] The administration instructions state that the reconstituted solution should be administered immediately, and that any unused reconstituted material should be discarded.[14]

27. Fertinex was a single-use product that needed to be reconstituted each time it was to be used.

28. Dosing with Fertrinex occurred daily for at least seven days using from one to four (or more) vials per day, followed by a dose of human chorionic gonadotropin (hCG),[15] which is also a heterodimeric gonadotropin.

29. As mentioned elsewhere herein, most if not all hCG products on the market when Fertinex was being developed and marketed contained a preservative (usually benzyl alcohol) and were multi-dose products.

30. Even though the recommended course of therapy with Fertinex required multiple dosings over many days, and even though Serono, the company that manufactured and marketed Fertinex, also manufactured and marketed an hCG product (Profasi) in

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13 PDR, 55th Ed., 2001, pages 3020 – 3022 (Reference CAY).

14 Id., page 3022.

15 Id.

a preserved multi-dose form during the period that Fertinex was being developed and marketed, Serono never provided Fertinex as a more convenient, multi-dose product prior to the present invention.

Gonal – F

31. Serono marketed an FSH product called Gonal-F beginning in about 1996. Gonal-F was a human FSH preparation of recombinant DNA origin in the form of a sterile, lyophilized powder containing r-FSH, sucrose, and phosphate salts.[16] The user was instructed to dissolve the contents of an ampule of Gonal-F in Sterile Water for Injection, U.S.P., to administer subcutaneously immediately, and to discard any unused reconstituted material.[17] Gonal-F was an FSH product that did not contain a preservative from the time of launch in about 1996 until the time that the present invention was made.

32. Gonal-F was a single-use product that needed to be reconstituted each time it was to be used.

33. Dosing with Gonal-F occurred daily for at least seven days using from one to four (or more) vials per day, followed by a dose of human chorionic gonadotropin (hCG),[18] which is also a heterodimeric gonadotropin.

34. As mentioned elsewhere herein, most if not all hCG products on the market when Gonal-F was being developed and marketed contained a preservative (usually benzyl alcohol) and were multi-dose products.

35. Even though the recommended course of therapy with Gonal-F required multiple dosings over many days, and even though Serono, the company that manufactured and marketed Gonal-F, also manufactured and marketed an hCG product (Profasi) in

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16 PDR, 54th Ed., 2000, pages 2943 – 2946 (Reference CAZ).

17 Id., page 2945.

18 Id.

a preserved multi-dose form during the period that Gonal-F was being developed and marketed, Serono never provided Gonal-F as a more convenient, multi-dose product prior to the present invention.

Follistim

36. Organon marketed an FSH product called Follistim beginning in about 1997. Follistim contained hFSH manufactured by recombinant DNA technology that was presented as a sterile, freeze-dried cake containing FSH, sucrose, sodium citrate, and polysorbate 20.[19] The user was instructed to dissolve the freeze-dried cake using Sterile Water for Injection, U.S.P., to administer the reconstituted Follistim immediately, and to discard any unused reconstituted material.[20] Follistim was an FSH product that did not contain a preservative from the time of launch until the time that the present invention was made.

37. Follistim was a single-use product that needed to be reconstituted each time it was to be used.

38. Dosing with Follistim occurred daily for up to 14 days, followed by a dose of human chorionic gonadotropin (hCG),[21] which is also a heterodimeric gonadotropin.

39. As mentioned elsewhere herein, most if not all hCG products on the market when Follistim was being developed and marketed contained a preservative (usually benzyl alcohol) and were multi-dose products.

40. Even though the recommended course of therapy with Follistim required multiple dosings over a period of up to 14 days, and even though Organon, the company that manufactured and marketed Follistim, also manufactured and marketed an hCG product (Pregnyl) in a preserved multi-dose form during most of the period that

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19 PDR, 54th Ed., 2000, pages 2092 – 2095 (Reference CBA).

20 Id., page 2094.

21 Id., page 2095.

Follistim was being developed and marketed, Organon never provided Follistim as a more convenient, multi-dose product before the present invention.

Summary – FSH Products

41. Many FSH products were on the market or were introduced over a period of more than thirty years prior to the present invention. None of these products were multi-dose products, and none contained a preservative, despite the fact that all were intended to be administered multiple times to each patient who received them.

hCG PRODUCTS

I further declare that:

42. In sharp contrast, preservatives had been used in products containing a protein that is related structurally to FSH, namely, chorionic gonadotropin (CG or hCG). Like FSH, chorionic gonadotropin is comprised of an α-subunit and a β-subunit. Human FSH and human chorionic gonadotropin are comprised of the same α-subunit. The two proteins differ markedly; notably, in the amino acid sequences of their β-subunits and hCG has a C-terminal extension that FSH lacks. HCG's C-terminal extension contains several O-linked glycosylation sites.

43. At the time that the presently claimed invention was made, each of the following hCG products had contained benzyl alcohol as a preservative for many years, as many as about 35 years in one case.

A.P.L.

44. Ayerst first marketed APL in the 1950s.[22] In 1965, APL was provided in both solution form and in dry form.[23] The solution product contained chorionic gonadotropin, sodium chloride and 0.5% phenol. In the dry form, chorionic

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22 PDR, 12th Ed., 1957, page 625 (Reference CBB).

23 PDR, 19th Ed., 1965, page 527 (Reference CBC).

gonadotropin was to be reconstituted with a sterile diluent resulting in a solution that also contained 2.0% benzyl alcohol and lactose.[24] APL was for intramuscular injection only.

45. In 1997, APL contained hCG, lactose, 2.0% benzyl alcohol, and not more than 0.2% phenol, once reconstituted.[25] APL could be stored for 30 days in a refrigerated after being reconstituted.

46. APL was an hCG product that contained benzyl alcohol from at least as early as 1965 until the time that the present invention was made.

Profasi

47. Serono began to market an hCG product, called Profasi, before 1980. Serono still marketed Profasi in 1997, at which time the product still provided human chorionic gonadotropin in lyophilized multiple dose vials together with a vial of Bacteriostatic Water for Injection containing 0.9% benzyl alcohol.[26] The 1997 PDR states that after reconstititing, Profasi needed to be refrigerated, and had to be used completely within 30 days.[27]

48. Profasi was an hCG product that contained benzyl alcohol from at least as early as 1980 until the time that the present invention was made.

Pregnyl

49. Organon marketed an hCG product, called Pregnyl, from before 1985.[28] When the freeze-dried hCG was reconstituted with Bactiostatic Water for Injection which was provided as a diluent, the resulting solution contained hCG, mannitol, phosphate salts,

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24 Id.

25 PDR, 51st Ed., 1997, page 2805 (Reference CAO).

26 PDR, 51st Ed., 1997, pages 2620 – 2621 (Reference CAQ).

27 Id., page 2621.

28 PDR, 39th Ed., 1985, page 1450 (Reference CBE).

and 0.9% benzyl alcohol.[29] After reconstituting, Pregnyl was to be refrigerated and used within 60 days.[30]

50. In 1997, Organon still marketed Pregnyl.[31] When reconstituted, Pregnyl contained hCG, phosphate salts, sodium chloride, and 0.9% benzyl alcohol.[32] After reconstituting, Pregnyl was to be refrigerated and used within 60 days.[33] The PDR also states that the "RECONSTITUTED SOLUTION IS STABLE FOR 60 DAYS WHEN REFRIGERATED" and "[r]econstituted material will remain stable for 60 days when refrigerated."[34]

51. Pregnyl was an hCG product that contained benzyl alcohol from at least as early as 1985 until the time that the present invention was made.

Other hCG Products

52. Several other hCG products had been on the market, including Progon, Stemutrolin, Glukor, and Follutein.

53. The administration and dosing instructions for Progon required administration of 125 to 500 IU per injection, and the product was provided in vials containing either 5,000 IU or 10,000 IU of hCG.[35] While it is not certain that Progon contained a preservative, the product was probably a multi-dose product that contained a preservative.

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29 Id.

30 Id.

31 PDR, 51st Ed., 1997, page 1878 (Reference CAP).

32 Id.

33 Id.

34 Id.

35 PDR, 19th Ed., 1965, page 646 (Reference CBF).

54. Glukor was supplied in vials of 10 cc and 25 cc, whereas, 1 cc was to be administered once or twice a week.[36] While it is not certain that Glukor contained a preservative, the product was probably a multi-dose product that contained a preservative.

55. Stemutrolin was a multiple dose product that contained hCG, urea, phosphate salts, and 1.5% benzyl alcohol after reconstitution with a diluent.[37]

56. Follutein was a multiple dose product that contained hCG, sodium chloride and 0.5% phenol as a preservative after reconstitution with a sterile aqueous diluent.[38] Once reconstituted, Follutein was supposed to be stored in a refrigerator, and when refrigerated, "the solution retains its potency for 2 months, thereafter potency slowly diminishes."[39]

Summary – hCG Products

57. For more than 40 years before the present invention was made, hCG products were multi-dose products that were preserved. The most frequently used preservative was benzyl alcohol.

58. During this lengthy period in which most hCG products contained a preservative, all human FSH products were NOT preserved.

59. All the FSH products marketed in this period by the two companies that also marketed hCG products were NOT preserved.

OTHER PROTEIN PRODUCTS

I further declare:

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36 PDR, 19th Ed., 1965, page 844 (Reference CBG).

37 PDR, 24th Ed., 1970, page 867 (Reference CBH).

38 PDR, 24th Ed. 1970, pages 1249 –1250 (Reference CBI).

39 Id., page 1250.

60. During the period when FSH products were marketed as single-use products without preservatives, that is, from the 1960s through the time that the present invention was made, several other protein products were available as multi-dose products with preservatives.

Insulin and glucagon

61. Lilly and Novo marketed numerous, multi-dose formulations of insulin and glucagons, most often preserved with phenol and/or m-cresol.[40]

Growth Hormone

62. Serono marketed a multi-dose, growth hormone product in the 1980s called Asellacrin that could be reconstituted from a lyophilized powder using Bacteriostatic Water for Injection.[41] Bacteriostatic Water for Injection contained a preservative, though the specific preserverative was not specified.

63. Lilly launched a multi-dose, growth hormone product in about 1986 called Humatrope that contained somatropin, mannitol, glycine, phosphate salts, glycerin, and 0.3% m-cresol after reconstitution.[42] After reconstituting, Humatrope needed to be stored refrigerated (2 °C – 8 °C), and used within 14 days.[43] Humatrope was on the market when the present invention was made.

64. Genentech launched a multi-dose growth hormone product in the mid-1980s called Protropin that contained somatrem, mannitol, phosphate salts, and 0.9% benzyl

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40 PDR, 19th Ed., 1965, page 703 – 704, 704 - Glucagon (Reference CBJ) and page 705 – Regular Iletin (Reference CBK).

41 PDR, 34th Ed., 1980, pages 1605 – 1606 (Reference CBL) and PDR, 39th Ed., 1985, pages 1940 – 1941 (Reference CBM).

42 PDR, 44th Ed., 1990, pages 1216 – 1217 (Reference CBN).

43 Id.

alcohol after reconstitution.[44] After reconstituting, Protropin needed to be stored refrigerated (2 °C – 8 °C), and used within 14 days.[45]

65. Serono launched a multi-dose growth hormone product in about 1990 called Saizen that contained somatropin, sucrose, phosphoric acid, and 0.9% benzyl alcohol after reconstitution.[46] The reconstituted solution was supposed to be stored refrigerated (2 °C – 8 °C) for up to 14 days.[47]

Erythropoietin products

66. Amgen launched a multi-dose, erythropoietin product called Epogen in about 1990 that contained epotein alfa, albumin, sodium citrate, sodium chloride, citric acid and 1% benzyl alcohol as preservative in Water for Injection.[48] The multi-dose solution was supposed to be stored refrigerated (2 °C – 8 °C), and used within 21 days after first entry of the vial.[49]

67. Ortho Biotech launched a multi-dose erythropoietin product called Procrit in about 1990 that contained epotein alfa, albumin, sodium citrate, sodium chloride, citric acid and 1% benzyl alcohol as preservative in Water for Injection.[50] The multi-dose solution was supposed to be stored refrigerated (2 °C – 8 °C), and used within 21 days after first entry of the vial.[51]

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44 PDR, 44th Ed., 1990, pages 1002 – 1003 (Reference CBO).

45 Id.

46 PDR, 52nd Ed., 1998, page 2776 – 2777 (Reference CBP).

47 Id, page 2777.

48 PDR, 51st Ed., 1997, pages 489 – 494, 489 (Reference CBQ).

49 Id., page 493.

50 PDR, 51st Ed., 1997, page 1896 (Reference CBR).

51 Id.

Conclusions – Other protein products

68. During the approximately thirty year period in which all FSH products were unpreserved, single-dose products, many other protein products were provided as multi-dose products containing preservatives, including benzyl alcohol.

I further declare that:

69. The fact that all of the FSH formulations discussed above were provided in lyophilized form for reconstitution immediately before use strongly suggested either that FSH was unstable in solution or that it could not be stably formulated with a preservative.

70. The Skrabanja patent[52] states that ". . . the stability of aqueous solutions of the gonadotropins is insufficient to allow storage for longer times. This is especially true for preparations containing the very pure gonadotropins, prepared using recombinant DNA methods, in relatively dilute solutions. Usually therefore those preparations are stored in a dry form, as is obtained after lyophilization." This statement strengthens the previous conclusion that the art strongly suggested that FSH was unstable in solution or that it could not be stably formulated with a preservative.

71. Attempts to stabilize FSH in solution have been published, such as Samaratini ("It is known that highly purified proteins are time-unstable and are stabilized, for instance, in admixture with saccharides, such as lactose and mannitol or else with proteins and amino acids, such as albumin and glycine.")[53] and Biome ("The single-chain forms of the heterodimers or homodimers have a number of advantages over their dimeric forms. . . . [T]hey are generally more stable.")[54] References such as these

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52 U.S. Patent No. 5,929,028, col. 2, ll. 21-30 (Reference AD).

53 U.S. Patent No. 5,650,390, col. 1, ll. 19-22 (Reference AF).

54 U.S. Patent No. 6,238,890, col. 4, ll. 18-20 (Reference AR).

demonstrate the instability of FSH, especially in solution formulations, and the extent to which scientists had previously attempted to resolve the instability problem.

72. Considering that FSH had to be administered once or even twice a day over a period of up to 14 days, one skilled in the art would naturally have been strongly motivated for more than thirty years to develop, if possible, a multi-dose preserved product. Such a product would eliminate product waste and patient error in mixing, and increase patient safety and convenience. The fact that this long-standing problem was not solved earlier clearly suggests that combining benzyl alcohol and FSH would have presented issues preventing the development of a multi-use formulation, indicating that such a combination was not obviously going to be successful.

CONCLUSIONS

73. Looking backward from 1998 to the time when FSH products became available for use in humans in the 1960s, FSH products sharply contrast with many other injectable protein products that had to be administered multiple times in the course of treatment.

74. From the time that FSH products for humans were first launched in the 1960s, a patient receiving FSH needed multiple injections of the product over a period of several days, up to 2 weeks. Yet, no FSH product was suitable for use as a multi-dose product because none contained a preservative. This establishes a long-standing unsatisfied need for a preserved, multi-dose product.

75. Surprisingly, the same companies that made FSH products also made multi-dose, preserved hCG products. HCG is also a heterodimeric hormone containing an alpha subunit and a beta subunit. In addition, many other preserved, multi-dose protein products were available during the more than thirty years that FSH products were uniformly unpreserved, single-dose products.

76. Looking at the long-standing need for a multi-dose FSH product and the coincident, prolonged availability of preserved, multi-dose hCG products and other protein

products, a person skilled in protein formulation would have quite reasonably concluded that FSH was not be stable enough in the presence of preservatives to be a multi-dose product.

77. Based on the fact that: 1) there was a long-standing need for preserved FSH formulations for multi-dose use; 2) FSH had only been provided in lyophilized forms and had been suspected, reasonably, of being unstable in solution; and 3) despite the coincident and prolonged availability of preserved multi-dose hCG and other protein products, no one had published, used in public, or sold multi-dose, preserved, pharmaceutically acceptable formulations of FSH, I conclude that one skilled in the art was not motivated to combine the references cited by the Examiner and would not consider preserved formulations of FSH and benzyl alcohol to be obvious.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

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February 18, 2003

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EDUCATIONAL RECORD:

8/1977 to 5/1981	Loras College, Dubuque, Iowa B.S. Degrees in Biology and Chemistry GPA: 3.706, Magna Cum Laude Advisors: Dr. Edward T. Cawley and Dr. Kenneth Kraus Thesis: *"Water quality of the* Catfish Creek watershed, 1980; *An overview."*
8/1981 to 5/1987	University of Notre Dame, Notre Dame, Indiana Ph.D. Degree in Chemistry Field: Biochemistry GPA: 3.500 Advisor: Dr. Francis J. Castellino Thesis: *"I: Evaluation of the phosphatidylserine requirement of the intrinsic Factor X activating system. II. Homology and secondary structure in serine proteases."*

EXPERIENCE:

1979 Summer	Participant in the University of Illinois' Undergraduate Research Program in Engineering, Champaign, IL. *Program provided independent research in the area of fiber reinforced concrete.*
1980 Summer	Student Director of the 1980 Student-Oriented Studies Program, Loras College.

Responsibilities included management of scientific research team and financial resources.

8/1981 to 8/1982	Teaching Assistant, University of Notre Dame. *Responsibilities included instruction and supervision of undergraduate chemistry laboratories, administration and grading of examinations and laboratory reports.*
8/1983 to 4/1987	Research Assistant, University of Notre Dame. *Research focused on the intrinsic Factor X activating system in the bovine coagulation system.*
4/1987 to 7/1990	National Institute of Health Postdoctoral Fellow Cornell University, Ithaca, New York Field: Biophysical Chemistry Advisor: Dr. Harold A. Scheraga *Research focused on protein folding in bovine RNase A.*
9/1990 to 10/1994	Senior Pharmaceutical Chemist Eli Lilly and Company, Indianapolis, Indiana Parenteral Products Research and Development *Research has focused on protein-protein and protein-excipient interactions as well as manufacturing process unit operations investigations to determine their influence on protein formulations.*
10/1994 to 6/1997	Research Scientist Eli Lilly and Company, Indianapolis, Indiana Biopharmaceutical Product Development *Research has focused on protein-protein and protein-excipient interactions in formulations for marketed product support, manufacturing process unit operations and their influence on protein formulation stability for marketed product support, structural properties of proteins for NDA submission, and pre-formulation characterization of a new protein drug candidate.*
6/1997 to 1/1999	Research Scientist Eli Lilly and Company, Indianapolis, Indiana bioResearch Technologies and Proteins: Protein Optimization Team *Initial focus of my efforts was on the interviewing, hiring, and establishment of a world-class protein engineering team (25-members), referred to as the Protein Optimization Team. Research has focused on the rational development of a stable, new protein drug candidate for IND submission.*
1/1999 to Present	Senior Research Scientist, Group Leader Eli Lilly and Company, Indianapolis, Indiana

bioResearch Technologies and Proteins: Protein Optimization Team
Technical direction of research efforts on internal bioproduct opportunities associated with diabetes, inflammation, cardiovascular disease, dementia, autoimmune disease, and hematopoiesis. Efforts focused on engineering properties into bioproducts that enhance the pharmaceutical attractiveness and usefulness of the bioproduct. In addition, I supported the evaluation of potential in-licensing opportunities and contributed to legal support of numerous patents.

PUBLICATIONS:

Cummings, H. S., Ploplis, V. A., Beals, J. M., and Castellino, F. J. (1984). "Interspecies cross reactivity of monoclonal antibodies to various epitopes of human plasminogen", *Arch. Biochem. Biophys. 230,* 306-315.

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"Conformational studies of a peptide corresponding to a region of the C-terminus of ribonuclease A: Implications as a potential chain-folding-initiation-site", Beals, J. M., Proctor and Gamble, March, 1990, Cincinnati, Ohio.

"Conformational studies of a peptide corresponding to a region of the C-terminus of ribonuclease A: Implications as a potential chain-folding-initiation-site", Beals, J. M., Oregon State University, April, 1990, Corvalis, Oregon.

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"Pharmaceutical Opportunities for Biochemists", Beals, J. M., ACS Roadshow, Saginaw Valley State University, November, 1991, Saginaw, Michigan.

"Humulin R adsorption studies onto NPH crystals: An isothermal titration calorimetry study", Beals, J. M., and Dodd, S. W., Lilly Insulin Technical Conference, June 14, 1992, Fegersheim, FR.

"Adsorption of soluble insulin onto NPH insulin crystals: An electrostatic interaction", Beals, J. M., Dodd, S. W., Havel, H. A., Lakshminarayan, C., Redmon, M. P., Sargeant, C. M., Sullivan, G. R., and Kovach, P. M., Lilly Expo, Feburary, 1993, Eli Lilly & Co., Indianapolis, Indiana.

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"T—>R transition in 2Zn-insulin hexamer: Development of the algorithm for modeling the cooperative binding of preservative", Varshavsky, A. D., Saxberg, B. E. H., Dodd, S. W., and Beals, J. M., 48th Annual Calorimetry Conference, July, 1993, Durham, North Carolina.

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"Formulation Interactions in NPH Mixtures", Beals, J. M., Pharmaceutical Product Development Seminar Series, November, 1993, Eli Lilly & Co., Indianapolis, Indiana.

"NPH crystallization: The effect of [Zinc], [protamine], [Human Insulin], and pH on crystal size, crystal morphology, surface adsorption properties, and sedimentation

volumes", Beals, J. M., Sullivan, G. R., and Dodd, S. W., Lilly Insulin Technical Conference, March 7, 1994, Fegersheim, FR.

"Time-dependent NPH crystallization: A particle sizing and microscopy study", Beals, J. M., and Dodd, S. W., Lilly Insulin Technical Conference, March 7, 1994, Fegersheim, FR.

"Protein-protein and protein-ligand interactions in NPH formulations", Beals, J. M., March, 1994, Loras College, Dubuque, Iowa.

"Pharmaceutical Opportunities for Biochemists", Beals, J. M., March, 1994, Loras College, Dubuque, Iowa.

"Adsorption of Soluble Insulin Hexamer onto the Surface of Insulin Crystals Cocrystallized with Protamine", Beals, J. M., Dodd, S. W., Havel, H. A., Lakshminarayan, C., Redmon, M. P., Sargeant, C. M., Sullivan, G. R., and Kovach, P. M., August, 1994, 49th Annual Calorimetry Conference; Santa Fe, New Mexico.

"The structure of LysPro-insulin", Ciszak, E., Smith, G. D., Baker, J. C., Beals, J. M., Carter, N. D. and Frank, B. H., June, 1994, American Crystallographic Association, Atlanta, Georgia.

"Isothermal titrating calorimetry study of phenolic ligand binding in 2Zn- and 2Co-insulin hexamers in the presence of chloride ion", Beals, J. M., Birnbaum, D. T., Dodd, S. W., Varshavsky, A. D., and Saxberg, B. E. H., August, 1994, 49th Annual Calorimetry Conference; Santa Fe, New Mexico.

"Mathematical modeling and algorithms for phenolic ligand binding to insulin hexamer : Model refinement to optimize fit for reduced dimensionality", Varshavsky, A. D., Beals, J. M., and Saxberg, B. E. H., August, 1994, 49th Annual Calorimetry Conference; Santa Fe, New Mexico.

"Adsorption of soluble insulin hexamer onto the surface of insulin crystals cocrystallized with protamine", Beals, J. M., Dodd, S. W., Havel, H. A., Lakshminarayan, C., Redmon, M. P., Sargeant, C. M., Sullivan, G. R., and Kovach, P. M., October 21, 1994, Bioprocess Research and Development Seminar Series, Eli Lilly & Co., Indianapolis, Indiana.

"The Structure of Lys^{B28}-Pro^{B29}-Insulin", Frank, B. H, Baker, J. C., Beals, J. M., Carter, N. D., Ciszak, E., Pekar, A. H., and Smith, G. D., November 6, 1994, International Diabetes Federation, Kobe Japan.

"Hierarchical modeling of phenolic ligand binding in 2Zn– and 2Co–insulin hexamers: Enthalpy and entropy compensation considerations", Birnbaum, D. T., Dodd, S. W., Saxberg, B. E. H., Varshavsky, A. D., and Beals, J. M., March 21, 1995, Computational Chemistry Seminar Series, Eli Lilly & Co., Indianapolis, Indiana.

"Formation and Disruption Kinetics of Cobalt-Insulin Hexamers: Ligand/Anion Binding and Cooperativity", Birnbaum, D. T., Kilcomons, M. A., Beals, J. M., and DeFelippis, M. R., July 8-12, 1995, 9th Symposium of The Protein Society, Boston, Massachusetts.

"Crystal Structure of the obese Protein Glu^{100}hOB", Zhang, F., Basinski, M. B., Beals, J. M., Briggs, S. L., Churgay, L. M., Clawson, D. K., DiMarchi, R. D., Furman, T. C., Hale, J. E., Hsiung, H. M., Schoner, B. E., Smith, D. P., Zhang, X. Y., Wery, J.-P., Schevitz, R. W., July 19-25, 1997, American Crystallization Association Meeting, St. Louis, Missouri.

"Structural Studies of LisPro-insulins", Ciszak, E., Frank, B. H., Beals, J. M., Yip, C. M., DeFelippis, M. R., Smith, G. D., July 19-25, 1997, American Crystallization Association Meeting, St. Louis, Missouri.

"Structure and Function of LysPro–Insulin: Insights Discovered During Development and Commercialization", Beals, J. M., DeFelippis, M. R., and Frank, B. H., May 19, 1995, Grand Rounds Seminar Series, Eli Lilly & Co., Indianapolis, Indiana.

"Reversible Adsorption of Soluble Hexameric Insulin onto the surface of Insulin Crystals Cocrystallized with Protamine: An Electrostatic Interaction", Dodd, S. W., Havel, H. A., Kovach, P. M., Lakshminarayen, C., Redmon, M. P., Sargeant, C. M., Sullivan, G. R., and Beals, J. M., May 22, 1995, American Association of Pharmaceutical Scientists Midwest Regional Meeting, Chicago Illinois.

"Structure and Function of LysPro–Insulin", Beals, J. M., June 8-9, 1995, Diabetes Care Global Conference: Approaching Physiologic Control of Blood Glucose in Patients with Diabetes Mellitus, Eli Lilly & Co., Indianapolis, Indiana.

"The Development of LY355101: A Story Explored, in part, by a Protein Iteration Philosophy." Beals, J. M. and Wery, J.-P., March 1997, Grand Rounds Seminar Series, Eli Lilly & Co., Indianapolis, Indiana.

"A Researcher's View of Statistical Design of Experiments for Assessing Biophysical Properties of Biopharmaceutical Formulations", Beals, J. M., May 19-21, 1997, Midwest Biopharmaceutical Statistics Workshop, Muncie, Indiana.

"Receptor Binding Kinetics of Erythropoietin are Affected by Glycosylation: Role of Electrostatic Interactions", Darling, R. J., Kuchibhotla, U., and Beals, J. M., February 10-15, 2002, Gordon Research Conference on Reversible Associations in Structural and Molecular Biology, Ventura, California.

"Understanding the Role of Electrostatics in Erythropoietin Receptor/Ligand System", Beals, J. M., Darling, R. J., and Kuchibhotla, K., April 19, 2002, Loras College, Dubuque, Iowa.

"Scientific Career Opportunities at Lilly", Beals, J. M., April 19, 2002, Loras College, Dubuque, Iowa.

"Understanding the Role of Electrostatics in Erythropoietin Receptor/Ligand System", Beals, J. M., Darling, R. J., and Kuchibhotla, K., June 6, 2002, Keynote Talk at the University of Notre Dame Chemistry and Biochemistry Annual Retreat, Pokagon Sstate Park, Algona, Indiana.

"Scientific Career Opportunities at Lilly", Beals, J. M., June 6, 2002, University of Notre Dame Chemistry and Biochemistry Annual Retreat, Pokagon State Park, Algona, Indiana.

"Understanding the Role of Electrostatics in Erythropoietin Receptor/Ligand System", Beals, J. M., Darling, R. J., and Kuchibhotla, K., October 15, 2002 , Loras College, Dubuque, Iowa.

"Careers in Biochemistry", Beals, J. M., October 15, 2002, 2002 ITAG Young Scholars' Conference, Loras College, Dubuque, Iowa.

PATENTS

Numerous Patent Applications Filed

HONORS:

Scholarship Recipient - Loras College
Member of Delta Epsilon Sigma Honor Fraternity, Alpha Chapter - Loras College
Participant in National Science Foundation's Review Board for Undergraduate Research Programs, Denver, CO - Loras College, 1977
Presiding Member at the National Science Foundation's Winter Meeting for the Student-Oriented Studies Programs, Washington, D. C. - Loras College, 1978
American Heart Association's Paul A. Nicoll Fellowship - University of Notre Dame, 1984-1985
Arthur J. Schmitt Dissertation Year Fellowship - University of Notre Dame, 1985-1986
Rohm and Haas Graduate Student of the Year in Chemistry - University of Notre Dame, 1985-1986
National Institute of Health Postdoctoral Fellowship - Cornell University, 1987-1990
2500-Day Product Development Award - Eli Lilly & Co., 1995
Lilly Research Laboratories President's Recognition Award, 1995
Lilly Research Laboratories President's Recognition Award, 1997
Lilly Change the World Award, Q3'2002

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New York Academy of Science

REFERENCES:

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Additional references available upon request.

POSTDOCTORAL RESEARCH SUMMARY:

The principle aim of my research at Cornell focused on the identification of potential chain-folding-initiation-sites (CFIS) and studying their contribution to the refolding of Ribonuclease A (RNase A). My work utilized a tryptic digest fragment identified as O-T-16, a C-terminal 20 amino acid peptide of RNase A. Theoretical predictions suggest that this hydrophobic region of RNase A is a potential CFIS and possibly dictates the intramolecular interaction necessary for rapid folding of RNase A. My results indicated the potential for a compact structure in O-T-16, with limited structural distributions observed under folding conditions.

My approach to this problem employed nonradiative energy transfer (NET) to ascertain distance constraints, from 15-70 Å, in O-T-16 under folding and unfolding conditions. This approach necessitated that site specific, stoichiometric chemical modifications be made to the peptide. The labeling involved extensive use of reverse-phase and ion-exchange HPLC. The interprobe distances were determined by measuring the effects of nonradiative energy transfer on the fluorescence of the donor using both steady-state and lifetime decay experiments. The distance distributions in the structures, determined from the initial NET results, indicated that hydrophobic bonds play significant role in the expression of structure in this peptide under folding conditions.

My primary purpose for doing postdoctoral research in the area of protein folding was to learn more about protein structure in an attempt to assimilate and apply this information in the areas of protein-protein and protein-lipid interactions.

GRADUATE THESIS RESEARCH SUMMARY:

The principle aim of my graduate research at Notre Dame was to investigate the roles that acidic phospholipid, the protein cofactor, factor $VIII_a$, and protein structure of factors IX_a and X play in the assembly of the bovine intrinsic tenase complex. The study was an integrated one utilizing techniques used in both lipid and protein chemistry. My results showed that 1) domains of phosphatidylserine (PS) on the lipid surface are not necessary

for vitamin-K dependent binding, 2) binding of factor IX_a and its activation products are PS concentration dependent, 3) two different kinetic models are required to explain activation of factor X in the presence and absence of factor $VIII_a$, and 4) the identification of factors IX and X as a+b proteins with secondary structure models determined using circular dichroism and a combined predictive algorithm.

The research approach required the isolation of blood coagulation factors VIII, IX, X, and XI purified to homogeneity using protein precipitation in addition to gel-permeation, ion-exchange, and affinity chromatography. These coagulation factors were activated and stabilized for binding studies and kinetic assays of the reconstituted factor X activating complex.

Phospholipid vesicles composed of phosphatidylserine and phosphatidylcholine were used as a lipid source. Physical properties of vesicles were defined in an attempt to understand the roles these properties play in the assembly of the complex. Properties studied included vesicle size, composition, and inner/outer head group ratios utilizing techniques of quasi-elastic light scattering (QELS), high performance liquid chromatography (HPLC), and fluorescence spectroscopy, respectively.

Calcium mediated binding studies of numerous blood coagulation factors to defined vesicles were performed using dynamic light scattering. In addition, the physical size of various proteins when bound to vesicles were determined using QELS. The kinetics of the reconstituted complex were studied utilizing a continuous coupled colorimetric assay.

The secondary structures of the protease and substrate of the tenase complex were studied utilizing a two-dimensional approach involving circular dichroism and empirical calculations. This technique integrated computerized algorithms that resulted in the development of an empirical predictive program designed for both micro- and mini-computers.

Finally, work not related to the above study, involved the development and use of monoclonal antibodies to study structure and function relationships in plasminogen. In addition, the secondary structural elements of the kringle domains of plasminogen, tissue plasminogen activator, and prothrombin were determined.